

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application

Listing of Claims:

1. (Currently Amended) A method for determining the AZT susceptibility ~~the resensitization of a test HIV-1 RT enzyme to AZT~~, comprising:
 - a) providing a reaction well with the following reaction components comprising:
 - i. at least one template for an HIV-1 RT enzyme;
 - ii. at least one primer;
 - iii. at least one detectable dNTP substrate;
 - iv. AZT; and
 - v. at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
 - b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well the test ~~an HIV-1 RT enzyme chosen from a wild-type RT enzyme, and a mutant selected from the group consisting of M41L / M184V / T215Y; M41L / D67N / K70R / M184V / T215Y; M41L / D67N / K70R / M184V / L210W / R211K / L214F / T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS / T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1 BH-10,~~
~~wherein said test HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or AZT into the 3'-end of the at least one primer forming at least one new DNA strand that is complementary to and bound to said at least one template;~~
~~c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into bound to the template;~~

d) repeating steps b) through and c), replacing the wild type test HIV-1 RT enzyme with a mutant control HIV-1 RT enzyme that is known to be susceptible to AZT inhibition; and

e) comparing the amount of the detectable dNTP substrate measured from step c) with that from step d) determining the resensitization of HIV-1 to AZT by comparing the RT activity of the wild type RT enzyme with the RT activity of the mutant RT enzyme; wherein the resensitization of HIV-1 to AZT is determined the test HIV-1 RT enzyme is identified to be AZT resistant when the amount of the detectable dNTP substrate measured from step c) is more than that from step d); and the test HIV-1 RT enzyme is identified to be AZT susceptible when the amount of the detectable dNTP substrate measured from step c) is less than or equal to that from step d).

2. (Original) The method of claim 1, wherein the template is bound to the reaction well and is chosen from poly-rA or a heteropolymer RNA or DNA.
3. (Original) The method of claim 1, wherein the primer is chosen from oligo-dt or a primer that is complementary to the heteropolymer template.
4. (Original) The method of claim 1, wherein the detectable dNTP substrate is chosen from a radioactive labeled dNTP.
5. (Original) The method of claim 1, wherein the detectable dNTP substrate is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
6. (Original) The method of claim 1, wherein the detectable dNTP substrate binds to an optical tracer or a radioactive labeled tracer.
7. (Original) The method of claim 6, wherein the optical tracer is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
8. (Original) The method of claim 6, wherein the detectable dNTP precursor is bromodeoxyuridine-triphosphate.
9. (Original) The method of claim 7, wherein the optical tracer is a monoclonal anti-BrdU antibody, conjugated to alkaline phosphatase.

10 – 19. (Cancelled)

20. (Currently Amended) A method for identifying determining the effect of at least one mutation in an HIV-1 RT enzyme that increases or decreases the AZT susceptibility of on the resensitization of the HIV-1 RT enzyme to AZT, comprising:

- a) providing a reaction well with the following reaction components comprising:
 - i. at least one template for an HIV-1 RT enzyme;
 - ii. at least one primer;
 - iii. at least one detectable dNTP substrate;
 - iv. AZT; and
 - v. at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well a HIV-1 RT enzyme and a mutant selected from the group consisting of M41L / M184V / T215Y; M41L / D67N / K70R / M184V / T215Y; M41L / D67N / K70R / M184V / L210W / R211K / L214F / T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS / T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1_{BH-10}, wherein said HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or the AZT into the 3'-end of the at least one primer forming at least one new DNA strand that is complementary to and bound to at least one HIV-1 RT inhibitor into said at least one template;
- c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into bound to the template;
- d) repeating steps a) through c), in a new reaction well wherein the HIV-1 RT enzyme of step b) is chosen from at least one mutant RT enzyme replacing the HIV-1 RT enzyme with a mutant HIV-1 RT enzyme comprising at least

one mutation, wherein the mutant HIV-1 RT enzyme is otherwise identical to the HIV-1 RT enzyme except for the at least one mutation; and

e) comparing the amount of the detectable dNTP substrate measured from step c) with that from step d);

comparing the RT activity in the different reaction wells; and

~~f) determining the effect of the at least one mutation on the resistance of HIV-1 to AZT using step e);~~

wherein the at least one mutation increases the AZT susceptibility of the HIV-1 RT enzyme when the amount of the detectable dNTP substrate measured from step c) is more than that from step d); and the at least one mutation decreases the AZT susceptibility of the HIV-1 RT enzyme when the amount of the detectable dNTP substrate measured from step c) is less than that from step

~~d) effect of at least one mutation in an HIV-1 RT enzyme on the resensitization of HIV-1 to AZT can be determined.~~

21. (Currently Amended) A method for rapid screening the effects of mutations in an HIV-1 RT enzyme that increase or decrease the AZT susceptibility on resensitization of the HIV-1 RT enzyme to AZT, comprising:

- a) providing an array of reaction wells, each reaction well with the following reaction components comprising:
 - i. at least one template for an HIV-1 RT enzyme;
 - ii. at least one primer;
 - iii. at least one detectable dNTP substrate;
 - iv. AZT; and
 - v. at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to each reaction well a different

~~HIV-1 RT enzyme chosen from an wild-type HIV-1 RT enzyme or a mutant HIV-1 RT enzyme comprising at least one mutation, wherein the mutant HIV-1 RT enzyme is otherwise identical to the HIV-1 RT enzyme except for the at least one mutation, and a mutant selected from the group consisting of M41L / M184V / T215Y; M41L / D67N / K70R / M184V / T215Y; M41L / D67N / K70R / M184V / L210W / R211K / L214F / T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS / T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1_{BH-10},~~

~~wherein said HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or the AZT into the 3'-end of the at least one primer forming at least one new DNA strand that is complementary to and bound to said template and wherein at least one wild-type HIV-1 RT enzyme is added to at least one reaction well of the array of reaction wells;~~

- c) ~~determining RT activity in each reaction well by measuring the amount of the detectable dNTP substrate incorporated into bound to the template; and~~
- d) ~~comparing the amount of the detectable dNTP substrate measured from the well containing the mutant HIV-1 RT enzyme with that from the well containing the HIV-1 RT enzyme determining the effect of mutations on resensitization of HIV-1 of the AZT by comparing the RT activity of at least one wild-type RT enzyme with the RT activity of at least one mutant RT enzyme;~~

~~wherein mutations that increase or decrease the AZT susceptibility of the HIV-1 RT enzyme are rapidly identified. the rapid screening the effects of mutations on resensitization of HIV-1 to AZT is determined. -~~

22. (New) The method of claim 1, wherein the control RT enzyme in step d) is a wild-type HIV-1 RT enzyme.

23. (New) The method of claim 20, further comprising the steps of:

- f) repeating step d) of the method of claim 20, omitting the at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- g) comparing the amount of the detectable dNTP substrate measured from step f) with that from step c) of claim 20, and with that from step d) of claim 20; wherein the at least one test mutation can be resistant to other HIV inhibitor(s) in addition to AZT when the amount of the detectable dNTP substrate measured from step f) is more than that from step c) of claim 20, and is substantially the same as that from step d) of claim 20.